Studies on Some Thiophene and Anthracenoid Compounds

by

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THE UNIVERSITY OF ALBERTA

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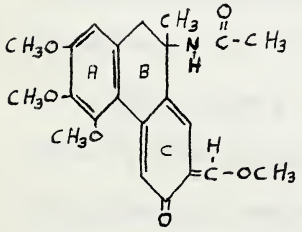
INTRODUCTION

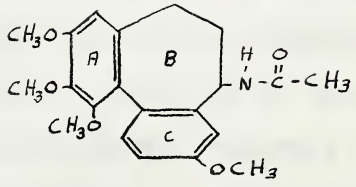
Colchicine is a very poisonous alkaloid, and this fact has led to a study of its action on cancerous tumors. The original structure for colchicine (Windaus)[1] is shown to have ring B consist of six members. The Windaus structure has been shown to be in error by Cohen, Cook and Roe[2] and they have suggested the correct structure to have rings B and C consist of seven members.

Downing, Hartwell, Leiter and Shear[3] have found that a number of compounds analogous to colchicine are potent in damaging Sarcoma 37 (a form of mammalian cancer).

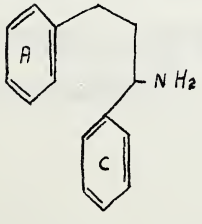
Lettré and Feinholz[4] have prepared a number of open chain derivatives of the original six membered structure (Windaus) and found that these compounds possess the ability to inhibit the mitosis of certain cells in tissue culture. Hartwell and coworkers have also synthesized some substituted α, β, diphenylethylamines[5] and found them to have the ability to damage Sarcoma 37.



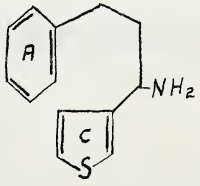
Colchicine (Windaus)



N-acetylcolchicine methyl ether



Diphenylpropylamine



1-(3-thienyl)-3-phenyl propylamine-1

Based on the structure of a compound such as colchinol methyl ether in which ring B is a seven membered ring, several alkoxyl substituted 1, 3, diphenylpropylamines have been prepared by Sandin and coworkers[6]. These compounds may be regarded as open chain analogues of colchicine.

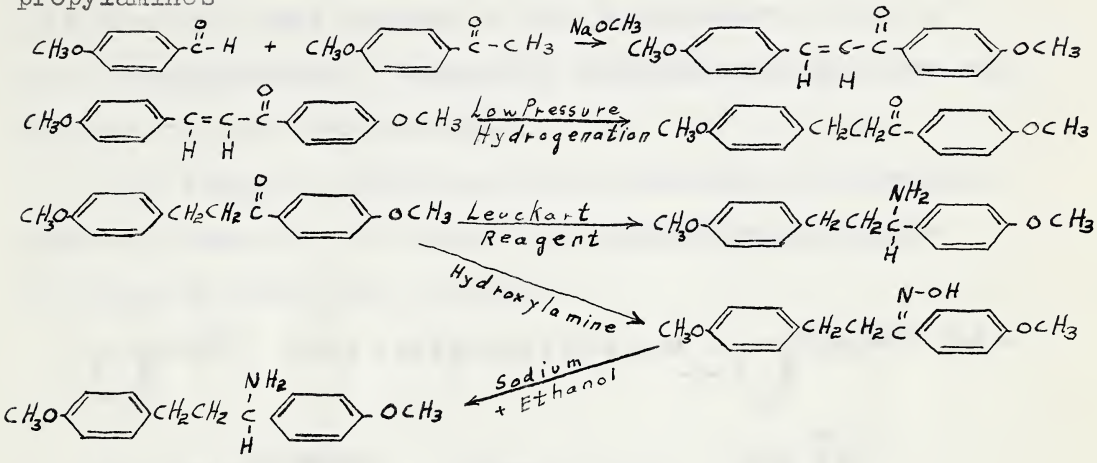
The effectiveness of these compounds in damaging Sarcoma 37 has been investigated by Hartwell and associates[7]. The results of their work showed that seven of the sixteen compounds tested showed good damaging activity on Sarcoma 37. However, in addition to this damaging effect, the compounds were also found to be quite toxic. The dosage required for physiological benefit was found to be very close to the maximum tolerated dose.

Campaigne recently investigated the effect of replacing a phenyl group in a pharmaceutical drug (phenolbarbital) by a thiophene group[8]. The 2-thienyl group in place of a phenyl group was found to possess reactivity and toxicity similar to that of the phenyl. However, the 3-thienyl group, when substituted for a phenyl gave a compound which appeared to be less toxic and more active biologically.

The primary objective of this work was to synthesize methoxy and methylenedioxy substituted 3-thienyl, phenyl, propylamines as well as ethylamines and methylamines. In addition an attempt was made to prepare the corresponding methoxy or methylenedioxy substituted phenyl, phenyl, propylamines.

DISCUSSION

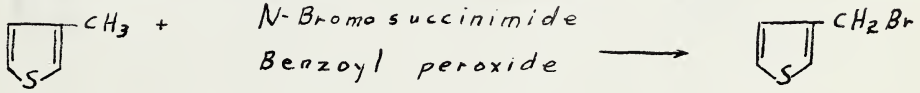
The following flow sheet indicates the reactions that have been used in the preparation of some 1-3 diphenyl propylamines[6]



It was hoped, although with some doubt, that a similar set of procedures could be used for the preparation of the corresponding thiopene compounds.

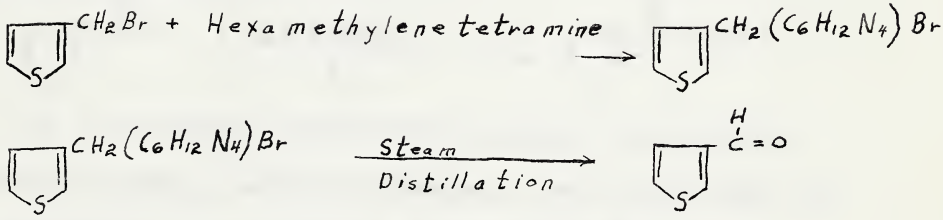
The 3-thiophene aldehydes and ketones were prepared according to the procedure of Campaigne and LeSuer[9]. The 3-methyl thiophene used in these reactions was obtained from the Winthrop laboratories and was used without further purification.

The first step in this series of reactions to obtain the desired aldehyde or ketone was the formation of 3-thenyl bromide.



This compound was prepared in good yield and a final vacuum distillation was not necessary as the crude material could be used in the preparation of 3-thenaldehyde. Work was done in a hood because of the lachrymatory effect of the 3-thenylbromide. Commercial N-Bromosuccinimide was used without further purification.

The 3-thenyl bromide was then converted to 3-thenaldehyde by formation of a salt with hexamethylenetetramine followed by steam distillation.

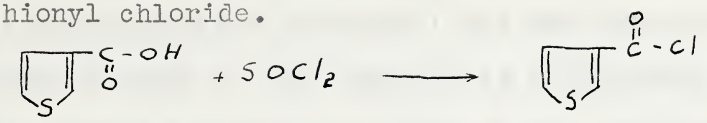


The purified 3-thenaldehyde was used directly in the preparation of a 3-thienyl substituted chalcone by condensation with p-methoxy acetophenone.

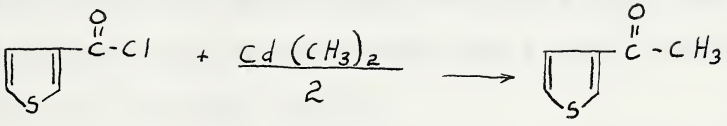
However, in order to prepare 3-thiophene substituted chalcones with the carbonyl group adjacent to the thiophene ring, 3-acetyl thiophene was prepared from 3- thenaldehyde. This was accomplished by a series of reactions[9].

The aldehyde was first converted to 3-thenoic acid by oxidation with silver oxide.

The crude acid was then changed to the acyl halide by pure thionyl chloride.

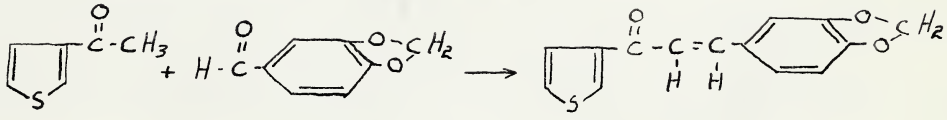


The 3-thenoyl chloride was easily purified by distillation at atmospheric pressure. 3-Acetyl thiophene was then prepared by the reaction of the acyl halide with dimethyl cadmium which was prepared from methyl magnesium bromide and cadmium chloride.



The 3-thiophene substituted aldehyde and ketone (3-thenaldehyde, 3 acetyl thiophene) were then used in the synthesis of 3-thiophene substituted chalcones.

Aldol condensations between the appropiate aldehyde and ketone gave the substituted chalcones.

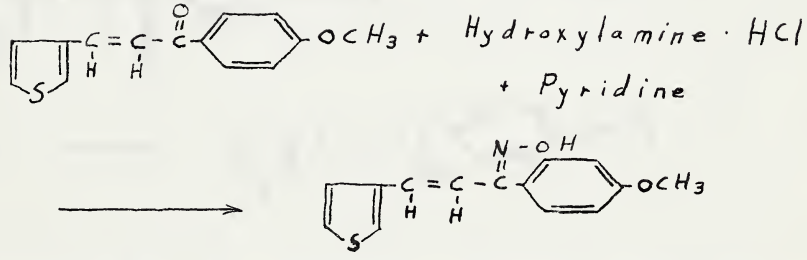


These compounds were easily obtained as yellow crystals and in good yield.

Attempts were then made to reduce the carbon-carbon double bond of these 3-thienyl substituted chalcones.

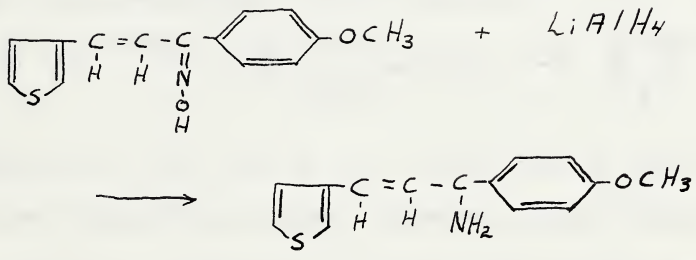
Low pressure hydrogenation (50 p.s.i.) was attempted using a platinum oxide catalyst[17] but was unsuccessful. The sulfur present in the compound as a thiophene ring probably caused a poisonous effect on the platinum oxide catalyst. The position of the carbonyl, whether adjacent to the thiophene ring or whether separated by a double bond, made no difference in the attempted hydrogenation of these compounds.

The thiophene substituted chalcones were then treated with hydroxylamine hydrochloride and a small amount of pyridine to yield the oximes.



The oximes were prepared in low yield and in both cases were obtained as yellow needles after recrystallization from ethanol. The low yield is probably due to the α, β, unsaturation in the chalcone which can result in by-product formation and thus decrease the yield. A weakly basic medium was found to be necessary for this oximination reaction.

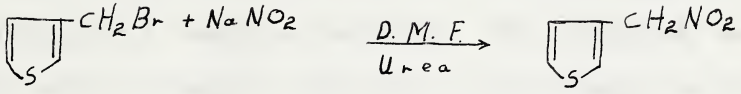
These oximes were then treated with an ethereal solution of lithium aluminum hydride with the intention of changing the oxime group to an amine group and possibly reducing the carbon-carbon double bond. In view of the fact that Gilsdorf and Nord[13] had reduced 1-(2-thienyl)-2-nitroethylene-1 to the 2-thiophene substituted ethylamine by treatment with lithium aluminum hydride, it did not seem unreasonable that the unsaturated oxime could be reduced to the saturated amine. However a carbon, hydrogen and sulfur analysis along with an infrared spectrum indicated that the double bond was still present in the final product.



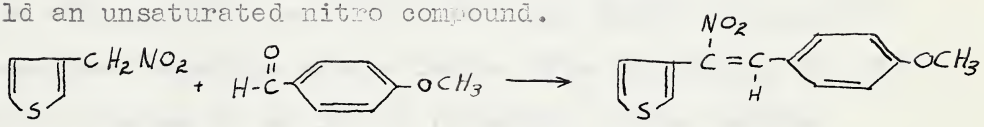
Due to the small amount of amine product obtained no further reactions were tried in order to reduce the double bond.

Sodium reducting agents i.e. sodium and ethanol or sodium amalgram could not be used because of the tendency for these reagents to desulfurize the compound being reduced.

A route to obtain a methoxy substituted 1-(3-thienyl)-2-phenyl ethylamine was also devised and attempted. For instance Kornblum and workers[11] have prepared some substituted nitromethane compounds by using the appropiate alkyl halide and sodium nitrite in a dimethyl formamide medium. 3-Thienyl nitromethane was prepared by this procedure.

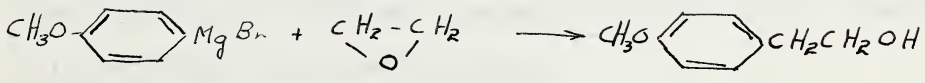


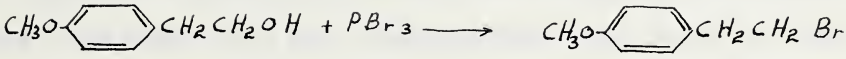
Yellow needles of this compound were prepared but in low yield. The next step in this proposed series of reactions would be to condense this nitro compound with an aldehyde to yield an unsaturated nitro compound.



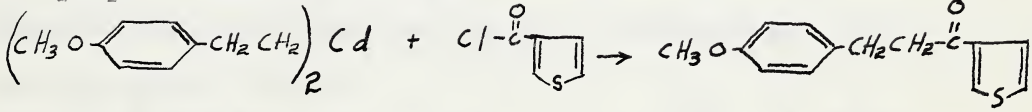
The next step was to be a reduction of this nitro compound to the corresponding amine. However, this route of synthesis still left the problem of a double bond reduction, and no reactions past the formation of the nitro compound were attempted.

Another series of reactions which would lead to the preparation of a saturated ketone, which could then be converted to an amine, was proposed. 1-p-Anisyl-2-ethanol was prepared according to the procedure of Speer and Hill[12] then reacted with phosphorous tribromide to yield the alkyl halide.



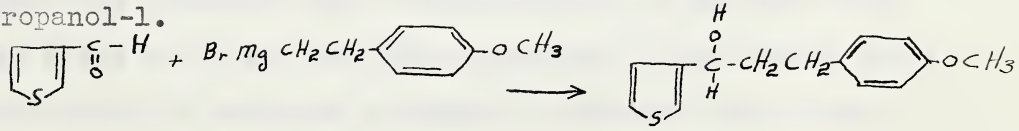


The Grignard reagent of 1-p-anisyl-2-bromoethane was then reacted with cadmium chloride in an attempt to prepare the dialkylaryl cadmium compound. This compound was then reacted with 3-thenoyl chloride in an attempt to prepare the proposed saturated ketone.



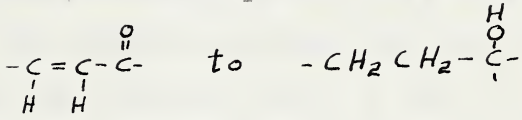
An oily material was isolated which failed to crystallize and gave negative ketone tests. The difficulty in this reaction appeared to be in the formation of the cadmium compound.

The synthesis of a saturated alcohol was then proposed, which could be converted to the amine by halogenation followed by a treatment with alcoholic ammonia. 1-p-anisyl-2-ethyl magnesium bromide was reacted with 3-thenaldehyde in the attempted preparation of 1-(3-thienyl)-3-(4-methoxyphenyl) propanol-1.



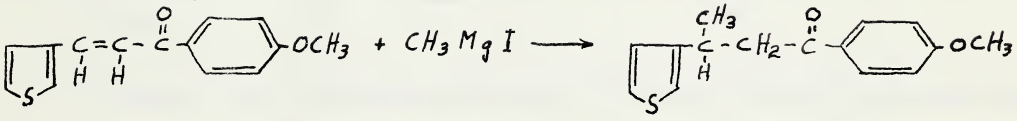
An oil was obtained from this reaction which contained a lower sulfur percentage than the calculated percentage. Upon standing a solid was isolated from the oil. However this material contained no sulfur.

Another attempt to reduce the original thiophene substituted chalcone was also investigated. By using sodium brohydride in a diglyme medium previous reports[14] had indicated that a reduction of:



was possible. This reaction was attempted, however, the product obtained was the unchanged starting material.

The reaction of methyl magnesium iodide with 3-thenylidene-p-methoxyacetophenone was also investigated. The reaction hoped for was:



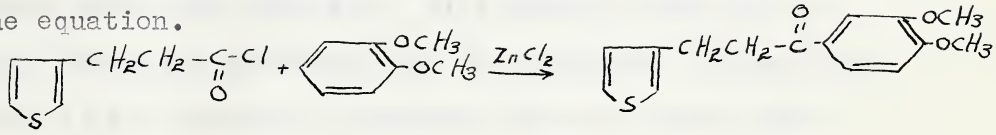
A small amount of oil was isolated but crystallization and attempts to prepare a derivative were unsuccessful.

Brown and Blanchette[15] have prepared 2, 5-dimethyl-3-thienyl-propionic acid by the reduction of 2, 5-dimethyl-3-thienylacrylic acid with sodium amalgram in a basic medium. For that reason the reduction of 3-thienylacrylic acid, which was prepared from 3-thenaldehyde and malonic acid, was tried out using the same procedure. After about eighteen hours of amalgram treatment a mixed melting point determination of the reaction product indicated that the reaction was incomplete. This material was therefore treated with more amalgram and heated until all the amalgram had decomposed. White needles were isolated but the melting

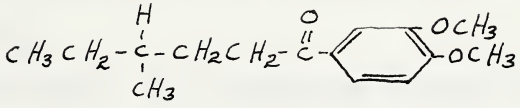
point was lower than the reported melting point. This material had a very disagreeable odor.

The material was then converted to the acyl halide by thionyl chloride. The product obtained from this reaction by vacuum distillation had a boiling point of 130° (25 mm.) The reported boiling point was 90-100° (3 mm.) and the compound was assumed to be 3-thienyl propionyl chloride.

The next step was the preparation of the ketone according to the equation.



However the compound obtained from the reaction contained no sulfur and preliminary carbon hydrogen analysis indicated a structure:

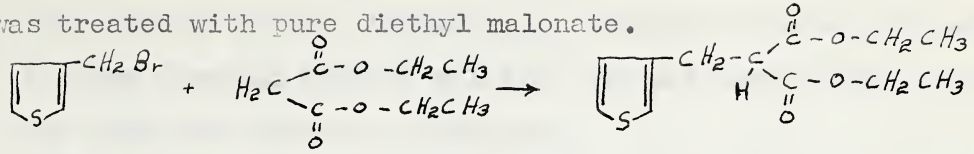


which would be like the compound afforded by a Raney nickel reduction. Stannic chloride was also tried as a catalyst in place of zinc chloride but immediate decomposition to a black benzene insoluble tar occured.

The results obtained seemed to indicate that desulfurization had occured during the sodium amalgram treatment and that the crude 3-thienyl propionyl chloride was in actual fact largely desulfurized material.

Another method of preparing 3-thienyl propionic acid devised by Campaigne[16] was then followed. 3-Thenyl bromide

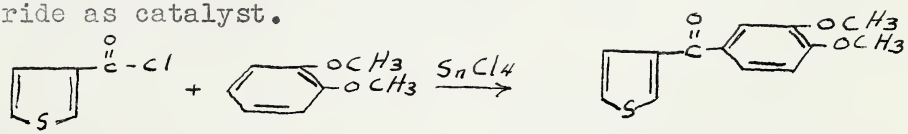
was treated with pure diethyl malonate.



The diethyl ester was saponified and decarboxylated to give a good yield of the acid. This 3-thienyl propionic acid was treated with thionyl chloride to yield the acyl halide in good yield.

The former reactions were then carried out with known 3-thienyl propionyl chloride. With stannic chloride as catalyst immediate decomposition was observed. Using zinc chloride as the catalyst, hydrogen chloride vapors were evolved and the reaction appeared to be successful. A small amount of oil was isolated from the reaction mixture but it failed to crystallize and attempts to prepare derivates were unsuccessful.

Finally 3-thienyl, 3, 4-dimethoxy phenyl ketone was prepared from 3-thenoyl chloride and veratrole using stannic chloride as catalyst.



This reaction proceeded without decomposition and a good yield was obtained.

A Leuckart reaction was attempted on 3-thienyl-3, 4-dimethoxy phenyl ketone but was unsuccessful.

The oxime of 3-thienyl 3, 4-dimethoxy phenyl ketone was prepared according to McElvain's procedure.[21] Attempts

were made to reduce the oxime to the amine with an ethereal lithium aluminum hydride solution but was unsuccessful and the oxime was recovered unchanged.

EXPERIMENTAL

3-Thenyl bromide.--To a three-necked flask fitted with an efficient stirrer, condenser and a short, wide bore funnel was added 100g. (1.02 mole) 3-methyl thiophene, 1.82g. (0.0075 mole) benzoyl peroxide and 300 ml. dry benzene. This mixture was brought to a vigorous reflux and the flame was removed. A powdered mixture of 160g. (0.89 mole) N-bromosuccinimide and 1.82g. (0.0075 mole) benzoyl peroxide was then added portionwise through the wide bore funnel as fast as violent foaming would permit. Addition usually required 20 minutes. The reaction flask was first cooled in cold water then in a ice-water mixture. The succinimide was filtered off and washed with dry benzene. The combined benzene extracts were evaporated at atmospheric pressure to remove most of the benzene. The crude material was used directly for the preparation of 3-thenaldehyde. The yield was about 80%.

3-Thenaldehyde.--Crude 3-thenylbromide, 100g. (0.565 mole) was added slowly with stirring to a mixture of 88g. (0.627 mole) hexamethylenetetramine in 225 ml. chloroform. The mixture was then heated on a water bath for thirty minutes. After cooling, 300 ml. water was added to the reaction mixture. The aqueous layer was separated from the chloroform layer, and the chloroform layer was extracted with two additional 100 ml. portions of water. The combined aqueous extracts were steam distilled. The cool distillate was extracted with several portions of ether. After drying over anhydrous sodium sulfate,

the ether was evaporated and the residue was distilled; yield about 60%, b. p. 190-193° (710 mm.), 80-81° (5 mm.), literature 195-199° (744 mm.)[9].

3-Thenaldehyde semicarbazone.--This compound was prepared according to the procedure of Shriner and Fuson[22]. The white solid was recrystallized from 60% ethanol and gave long white needles melting at 219-221°.

Anal. Calcd. for $C_6H_7N_3OS$: S, 18.84. Found: S, 18.91.

3-Thenylidene p-methoxyacetophenone.--To a cooled solution of 11.2g. (0.01 mole) 3-thenaldehyde and 15.0g. (0.10 mole) p-methoxyacetophenone dissolved in 100 ml. of methanol was added 2.0g. (0.087 mole) sodium dissolved in 50 ml. methanol. This mixture was allowed to stand twenty four hours. The resulting yellow crystals were filtered, washed with cold methanol and dried. Yield 22.7g. (93%). After recrystallization from ethanol the compound melted at 104-105°.

Anal. Calcd. for $C_{14}H_{12}O_2S$: C, 68.80; H, 4.95; S, 13.11. Found: C, 68.98; H, 5.05; S, 12.94.

Attempted preparation of β-3-thienyl p-methoxypropiophenone.--To a solution of 5g. (0.020 mole) 3-thenylidene p-methoxyacetophenone dissolved in 200 ml. ethanol was added 0.2g. Adam's platinum catalyst[17]. The reaction mixture

was placed in a low pressure hydrogenation apparatus and shaken for six hours. No pressure drop was observed. The reaction mixture was removed from the apparatus and the platinum black was filtered from the ethanol solution. After evaporation off of three quarters of the ethanol, the original compound crystallized out.

3-Thenylidene-p-methoxyacetophenone oxime.--3-Thenylidene-p-methoxyacetophenone 5g. (0.020 mole) and 6g. (0.087 mole) hydroxylamine hydrochloride in 200 ml. ethanol was refluxed for six hours. Most of the ethanol was evaporated off and the residue was poured on ice. The solid material was filtered off and recrystallized from ethanol. The pale yellow needles melted at 124-125°.

Anal. Calcd. for $C_{14}H_{12}O_2S$: S, 12.35. Found: S, 12.51, 12.11.

1-(3-Thienyl)-3-amino-3-(p-methoxyphenyl) propene-1.--To a slurry of 2g. (0.053 mole) $LiAlH_4$ in a three-neck flask with a stirrer, condenser and dropping funnel was added slowly 2g. (0.0077 mole) 3-thenylidene-p-methoxyacetophenone-oxime. The reaction mixture was refluxed for two hours and allowed to stand overnight. After refluxing for an additional one hour and standing for two hours, the excess $LiAlH_4$ was decomposed by careful addition of water. After 500 ml. of a 20% sodium potassium tartarate solution was added, the ethereal layer was separated and dried over sodium sulfate,

The ether was then evaporated off. This afforded white crystals which were recrystallized from an ethanol-water mixture, m.p. 92.5-94.5°.

Anal. Calcd. for $C_{14}H_{15}NOS$: C, 68.54; H, 6.16; S, 13.08. Found: C, 68.53; H, 6.35; S, 13.53, 13.34.

Benzylideneacetophenone.--To a cooled solution of 10.6g. (0.10 mole) distilled benzaldehyde and 12.0g. (0.10 mole) acetophenone dissolved in 100 ml. methanol was added a solution of 2.0g. (0.087 mole) sodium dissolved in 50 ml. methanol. The mixture was allowed to stand for twenty four hours. The resulting crystals were filtered off and washed with cold methanol. The melting point after recrystallization from ethanol was 54-57°; literature 57-58°[18].

Benzylideneacetophenone Oxime.--A mixture of benzylideneacetophenone 20g. (0.96 mole) and 12g. (0.173 mole) hydroxylamine hydrochloride, 5 drops of pyridine and 400 ml. ethanol were refluxed for two hours. Excess ethanol was evaporated off and the residue was poured on ice. The solid material was filtered off and after recrystallization from ethanol, the short, pale yellow needles melted at 113-115°; literature 115-116°[19].

β-Phenylpropiophenone.--To a solution of 10g. (0.048 mole) benzylideneacetophenone in 200 ml. ethanol was added 0.1g. Adams platinum catalyst. The reaction mixture was

subjected to low pressure hydrogenation for twelve hours. After the platinum black was filtered off, the filtrate was evaporated to one quarter of the original volume and then poured on ice. The solid which separated after recrystallization from ethanol afforded yellow crystals; m. p. 70-73°, literature 72-73°[20].

Attempted preparation of 1-3 diphenylpropylamine.-- Into a three-necked flask fitted with a tamworth condenser, stirrer and a dropping funnel was placed 5.0g. (0.135 mole) $LiAlH_4$ and 300 ml. dry ether. The mixture was stirred and refluxed for several hours. A solution of 10.8g. (0.05 mole) benzylideneacetophenoneoxime in 400 ml. dry ether was then added dropwise. Shortly after the termination of this addition, a few ml. of water were very carefully added to decompose the excess $LiAlH_4$. A 20% solution of sodium potassium tartarate (500 ml.) was then added to the mixture. The ethereal layer was separated and the aqueous solution was extracted with ether. The total ethereal extract was dried over sodium sulfate and the ether was evaporated off. This afforded a solid material which was recrystallized from ethanol and melted at 113-116°. A mixed melting point indicated this compound was the original oxime.

3-Thenoic acid (3-thiophenecarboxylic acid).--To 75g. (0.44 mole) of silver nitrate in 150 ml. water was added with continous stirring 35g. (0.88 mole) sodium hydroxide

in 150 ml. water. To this mixture, cooled in a ice-water bath, was added 25g. (0.22 mole) 3-thenaldehyde (crude) in small portions with stirring. The black silver suspension was filtered off and washed with two portions of hot water. The cold combined filtrate was acidified with concentrated hydrochloric acid. The precipitated 3-thenoic acid was filtered off and recrystallized from hot water; yield 12g. (70%), m. p. 138-139.5°, literature 137-138°[9].

3-Thenoyl chloride.--3-Thenoic acid 30g. (0.234 mole) was refluxed with 150g. (1.26 mole) thionyl chloride for one hour. The excess thionyl chloride was distilled off at atmospheric pressure. The residual 3-thenoyl chloride was distilled; yield 27.7g. (88%), b.p. 196° (700 mm.), m. p. 53-54°, literature m. p. 51-52°, b.p. 203-204° (748 mm.)[9].

3-Acetylthiophene.--In a three-necked flask fitted with condenser, stirrer and dropping funnel was placed 12g. (0.494 mole) magnesium and 400 ml. dry ether. Methylbromide was then passed into the mixture until all the magnesium had dissolved. To this Grignard reagent, cooled in a ice bath, was added slowly 45g. (0.246 mole) anhydrous cadmium chloride. The mixture was then removed from the ice bath and stirred for thirty minutes. After cooling in ice, 12g. (0.0823 mole) 3-thenoyl chloride dissolved in 40 ml. dry ether was added. Heat was applied

to initiate the reaction and refluxing was continued during the remainder of the addition of 24g. (0.165 mole) 3-thenoyl chloride and for one hour after completion of the addition. After cooling, the product was hydrolyzed with water and enough dilute sulfuric acid was added to dissolve the white precipitate. The ethereal layer was separated and the aqueous layer was extracted with ether. The combined ether extract was dried over sodium sulfate and the ether was evaporated off. The residual 3-acetylthiophene was distilled. yield 24.1g. (78%), m. p. 56-59°, literature 57°[9].

3, 4-Methylenedioxybenzylidene-3-acetylthiophene.--To 3-acetylthiophene, 3.3g. (0.026 mole) and 3.9g. (0.026 mole) piperonal in 25 ml. methanol was added 0.6g. (0.026 mole) sodium dissolved in 15 ml. methanol. This mixture was allowed to stand for several hours. The yellow crystals which formed melted at 125-127° after recrystallization from ethanol.

Anal. Calcd. for $C_{14}H_{10}O_3S$: S, 12.40. Found: S, 12.00.

Attempted preparation of 1-(3 thienyl)-3-(3, 4 methylenedioxyphenyl) propanone-1.--A low pressure hydrogenation was carried out on 3, 4-methylenedioxybenzylidene-3-acetylthiophene but there was no pressure drop and the starting compound was recovered unchanged. A platinum oxide catalyst was used.

3, 4-Methylenedioxybenzylidene-3-acetylthiophene oxime.--The oxime of 3, 4-methylenedioxybenzylidene-3-acetylthiophene was prepared according to the procedure of McElvain[21]. Yellow needles were recrystallized from ethanol and had a decomposition point of 162-167°.

Anal. Calcd. for $C_{14}H_{11}O_3NS$: C, 61.52; H, 4.06. Found: C, 60.72, H, 4.25.

1-(3, 4-Methylenedioxyphenyl)-3-(3-thienyl)-3-aminopropene-1.--To a slurry of 5g. (0.132 mole) $LiAlH_4$ (dissolved by refluxing for four hours) was added 7.7g. (0.028 mole) 3, 4-methylenedioxybenzylidene-3-acetylthiophene oxime. The reaction mixture was refluxed for two and one half hours. The excess $LiAlH_4$ was carefully decomposed by addition of water. The ether extract was dried with anhydrous sodium sulfate and the ether was evaporated off. After recrystallization from benzene the yellow crystals had a decomposition point of 170-172°.

Anal. Calcd. for $C_{14}H_{13}O_2NS$: S, 12.37. Found: S, 12.72, S, 12.64.

3-Thienyl nitromethane.--To a mixture of 150 ml. dimethyl formamide, 7.0g. (0.10 mole) sodium nitrite and 8.0g. (0.133 mole) urea was added 10g. (0.56 mole) of 3-thenyl bromide. The mixture was maintained at a temperature of 0-5° by a salt-ice bath and stirred for five hours. The reaction

mixture was then poured into 300 ml. petroleum ether. The petroleum ether layer was separated and the aqueous layer was extracted four times with petroleum ether. The solvent portion was dried with anhydrous sodium sulfate and then the solvent was removed by vacuum distillation. A fractional vacuum distillation was performed on the residue and material was collected from 40-140° (30 mm.). The total distillate was extracted with sodium hydroxide. The alkaline extract after acidification with sulfuric acid gave a solid, which after recrystallization from an ethanol-water solution gave yellow crystals, m. p. 136-139°.

Anal. Calcd. for $C_5H_5NO_2S$: S, 22.37. Found: S, 26.40.

1-p-Anisyl-2-ethanol.--The Grignard reagent of p-Bromoanisole was made by adding 45g. (0.24 mole) p-bromoanisole in 50 ml. dry ether to 5.76g. (0.24 mole) magnesium shavings. After the reagent had been cooled in an ice bath, a solution of 22g. (0.50 mole) ethylene oxide dissolved in 50 ml. dry benzene was added slowly with stirring. The mixture gelled before the addition was complete. The reaction mixture was allowed to stand a short time in a freezing bath and then was heated to 60° for three hours. The excess Grignard reagent was decomposed with dilute sulfuric acid. The benzene-ether solvent layer was separated, washed with water, a sodium carbonate solution and then dried over sodium sulfate. The solvent was evaporated off and the residue

was fractionally distilled, b. p. 165-190° (30 mm.), literature 145-160° (10 mm.)[12].

1-p-Anisyl-2-bromoethane.--To the crude 1-p-anisyl-2-ethanol 14.5g. (0.095 mole) dissolved in 50 ml. dry benzene was added 20g. (0.074 mole) phosphorous tribromide in small portions. After standing for thirty minutes the reaction mixture was heated on the steam bath till hydrogen bromide evolution ceased. The reaction mixture was poured on ice and the benzene layer was separated, washed with water and a sodium carbonate solution. After drying over sodium sulfate the benzene was evaporated off and the residue was fractionally distilled, b.p. 100-110° (2.3 mm.), yield 15.5g. (76%), literature 105-111° (1 mm.)[12].

Attempted preparation of 1-(3-thienyl)-3-(4-methoxyphenyl) propanone-1.--To a three-necked flask fitted with a stirrer, condenser and dropping funnel was placed 1.75g. (0.072 mole) magnesium in 200 ml. dry ether and to this 15.5g. (0.072 mole) 1-p-anisyl-2-bromoethane was added dropwise. The reaction was started by the use of iodine crystals and by heating. The reaction mixture was allowed to stand overnight. The solution was then cooled in an ice-water bath and 6.6g. (0.036 mole) cadmium chloride was added in small portions with stirring. The reaction mixture was then refluxed for thirty minutes. 3-Thenoyl chloride, 9.4g.

(0.072 mole) dissolved in 50 ml. dry ether was then added dropwise. The mixture was refluxed for two hours, then decomposed with water and dilute sulfuric acid. The ethereal layer was separated and the aqueous layer was extracted with ether. The total ethereal extract was dried over sodium sulfate and then the ether was evaporated off. The residue was made alkaline with sodium hydroxide then steam distilled. The distillate contained a white waxy material and the residue in the flask was a black tar. Both of these materials would not crystalize and both gave negative 2-4-dinitrophenyl-hydrazine tests.

Attempted preparation of 1-(3-thienyl)-3-(4-methoxy-phenyl) propanol-1.--The Grignard reagent of 1-p-anisyl-2-bromoethane was made by adding dropwise 10.5g. (0.046 mole) 1-p-anisyl-2-bromoethane to 1.13g. (0.046 mole) magnesium shavings in 200 ml. dry ether. 3-Thenaldehyde, 5.2g. (0.046 mole) in 50 ml. ether was then added and the mixture was refluxed for one hour then allowed to stand overnight. The reaction mixture was decomposed with water and then dilute sulfuric acid was added. The etheral layer was separated and dried over sodium sulfate and the solvent was evaporated off. The residue was fractionally vacuum distilled. A yellow oil distilling at 120-210° (5 mm.) was analyzed.

Anal. Calcd. $C_{14}H_{16}O_2S$: S, 12.89. Found: S, 8.60, S, 8.64.

Upon standing a solid was obtained from this yellow oil which after recrystallization from ethanol melted at 83-86°. However this compound contained no sulfur.

Attempted reduction of 3-thenylidene-p-methoxyacetophenone with sodium borohydride.--Sodium borohydride 1.11g. (0.03 mole) in 200 ml. diethyl diethyleneglycol was placed in a dry three-necked flask fitted with a stirrer, condenser and a drying tube. The mixture was stirred to effect a good solution. 3-Thenylidene p-methoxyacetophenone, 5g. (0.0205 mole) dissolved in diethyl diethyleneglycol was added to the mixture. Aluminum chloride 1.32g. (0.01 mole) dissolved in diethyl diethyleneglycol was then added slowly with stirring. After the exothermic reaction had subsided heating was done on a steam bath for one hour. The contents of the flask was then poured onto a mixture of crushed ice and 100 ml. concentrated hydrochloric acid. The organic solvent layer was separated, dried with sodium sulfate and the solvent was evaporated off. The solid obtained was recrystallized from ethanol and melted at 103-105°. A mixed melting point indicated that the final product was the original starting material.

Attempted preparation of 1-(p-methoxyphenyl)-3-methyl-3-thienylpropanone-1.--Methyl magnesium iodide was prepared by adding 5.0g. (0.035 mole) methyl iodide to 0.85g. (0.035 mole) magnesium in 200 ml. dry ether and refluxing till all

the magnesium had dissolved. To this solution was added slowly a saturated benzene solution of 5g. (0.205 mole) 3-thenylidene p-methoxyacetophenone. The excess Grignard reagent was decomposed with dilute sulfuric acid and the benzene-ether layer was separated. After drying over sodium sulfate the solvent was evaporated off. An orange oil remained which could not be crystallized from ethanol. Oximination was unsuccessful.

Attempted reduction of 3-thenylidene-p-methoxyacetophenone with $LiAlH_4$.--To a slurry of 2-3g. $LiAlH_4$ in dry ether was added an ethereal solution of 3g. (0.012 mole) 3-thenylidene-p-methoxyacetophenone. After standing for several hours the excess lithium aluminum hydride was decomposed by addition of water and dilute sulfuric acid. The ethereal layer was separated, dried over sodium sulfate and the solvent was evaporated off. The residue was a heavy yellow oil which failed to crystallize from ethanol.

3, 4-Dihydroxy-3-phenylpropiophenone.--To a mixture of 15g. (0.10 mole) hydrocinnamic acid and 22g. (0.20 mole) catechol was added 28g. (0.20 mole) anhydrous zinc chloride. The solution was maintained with stirring at 140-150° for one hour. The mixture was then poured into cold water and allowed to stand for two hours. The solid was filtered off and washed with water. The solid was recrystallized from an ethanol-water mixture, m. p. 150-151°.

Hydrocinnamoyl chloride.--To 15.0g. (0.10 mole) hydrocinnamic acid was added 23.6g. (0.20 mole) thionyl chloride. The solution was refluxed for two hours then the excess thionyl chloride was distilled off. The residue was vacuum distilled, b.p. 122° (20 mm.), yield 15.7g. (94%). literature 115-116° (11-12 mm.)[23].

3, 4-Dimethoxy-β-phenylpropiophenone.--To a solution of 6.9g. (0.05 mole) veratrole, and 8.4g. (0.047 mole) hydrocinnamoyl chloride in 150 ml. nitrobenzene was added with stirring 6.1g. (0.047 mole) powdered anhydrous aluminum chloride. This mixture was kept at 0° for three hours then allowed to slowly come to room temperature. The reaction mixture was then steam distilled and a yellow, nonvolatile residue was collected and was recrystallized from ethanol; yield 2.7g. (21%), m.p. 69-71°, literature 73°[24].

1-(3, 4-Dimethoxyphenyl)-3-phenylpropylamine-1.--To 10g. (0.037 mole) 3, 4-dimethoxy-3-phenylpropiophenone was added 20g. Leuckart reagent and the mixture was heated at 165-185° for five hours. The mixture was cooled and 2 to 3 volumes of water were added. The oil which separated was washed with more water then was heated for one hour with 25 ml. concentrated hydrochloric acid. The acidic layer was

decanted off and basified with sodium hydroxide, then extracted with benzene. The benzene extract was dried over sodium sulfate and the solvent was evaporated at reduced pressure. A residual oil (4.0g.) remained. The picrate was prepared by addition of picric acid to an ethanolic solution of the amine. The picrate was recrystallized from ethanol. m. p. 187-190°.

Anal. Calcd. for $C_{23}H_{24}O_9N_4$: C, 55.20; H, 4.83. Found: C, 54.88; H, 4.46.

3-Thienylacrylic acid.--3-Thenaldehyde 17.9g. (0.16 mole), 34g. (0.32 mole) malonic acid, 80g. dry pyridine and 1 ml. piperidine were warmed on a steam bath for three hours and then boiled for fifteen minutes. After cooling the solution was poured into water and treated with excess concentrated hydrochloric acid. The solid was filtered, washed with water and recrystallized from an ethanol-water mixture, m. p. 151-152°, yield 15.5g. (63%).

Anal. Calcd. for $C_7H_6O_2S$: S, 20.79. Found: S, 20.53, S, 20.87.

Attempted preparation of 3-thienylpropionic acid.--3-Thienylacrylic acid, 6.5 g. (0.042 mole) was just neutralized with a 5% sodium hydroxide solution and 200 g. of a 2% sodium amalgram (0.174 mole sodium) was added. The mixture was allowed to stand overnight then warmed on a steam bath

until all the amalgram had decomposed. The aqueous solution was decanted from the mercury, treated with norite and filtered hot. After cooling, the solution was acidified and the oil which separated solidified upon cooling in ice water. The solid was recrystallized from hot water as long white needles; yield extremely low, m. p. 50-55°, literature 61-62°[16].

Diethyl 3-thenylmalonate.--To 500 ml. absolute ethanol in a three-necked flask fitted with a condenser, stirrer and dropping funnel was added 23g. (1.0 mole) sodium. When all of the sodium had reacted 170g. (1.06 mole) diethyl malonate was added in a steady stream. Crude 3-thenyl bromide 150g. was added dropwise over a period of thirty minutes. The mixture was then refluxed with stirring for thirteen hours, cooled, and 450 ml. of a salt solution was added. The ester layer which separated was vacuum distilled. The product was collected from 140-198° (15 mm.), yield 71g. (38%), literature b.p. 146° (3 mm.)[16].

3-Thenylmalonic acid.--The diethylmalonate was saponified by refluxing with twenty percent aqueous sodium hydroxide for six hours. The reaction mixture was then acidified and extracted with ether. After evaporation of the ether, acidification and recrystallization from benzene there was obtained a white solid m. p. 139-140°, literature 138-139°[16].

3-Thenylpropionic acid.--All of the above 3-thenylmalonic acid was heated at 130-142° for one hour, then rapidly heated to 170°. The reaction mixture was then vacuum distilled and the fraction from 136-170° (30 mm.) was collected and allowed to cool. The solid material was filtered off and recrystallized from hot water, yield 15g. m. p. 60°, literature 61-62°[16].

β-3-Thienyl propionyl chloride.-- -3-Thienylpropionic acid 13.6g. (0.087 mole) and 20.8g. (0.172 mole) thionyl chloride was refluxed for ten minutes. After excess thionyl chloride was removed at reduced pressure the residue was vacuum distilled and the product was collected from 100-110° (30 mm.), 8.5g. (56%) of the product was obtained; literature 90-100° (3 mm.)[16].

Attempted preparation of β-3-thienyl (3, 4-dimethoxy)-propiophenone.--β-3-thienylpropionyl chloride 5g. (0.0287 mole) and 30g. (0.217 mole) veratrole was cooled to 0° in a ice-water bath. To this mixture was added portionwise 5g. (0.037 mole) anhydrous zinc chloride. The reaction mixture was stirred occasionally and allowed to stand overnight. The residue was poured into water and the mixture was steam distilled. An oil remained in the flask and was separated from the aqueous solution. Attempts were made to recrystallize the product from ethanol but only an oil was obtained. Attempts

to prepare an oxime drivative from this oil were also unsuccessful.

3-Thienyl-3, 4-dimethoxyphenyl ketone.--To a solution of 2g. (0.0136 mole) 3-thenoyl chloride and 3.8g. (0.027 mole) veratrole in 100 ml. dry benzene at 10° was added slowly and dropwise 1.8g. (0.007 mole) stannic chloride over a period of ninety minutes. The mixture was allowed to come to room temperature. The reaction mixture was decomposed by excess dilute hydrocloric acid and the benzene layer was separated, washed with a sodium carbonate solution then dried over sodium sulfate. The excess benzene was removed by evaporation at reduced pressure. About 1g. of residue remained which crystallized after standing two days. The white crystals were filtered off, dried and recrystallized from ethanol, m. p. 109-111°.

Anal. Calcd. for $C_{13}H_{12}O_3S$: S, 12.92. Found: S, 12.79, S, 12.64.

3-Thienyl 3, 4-dimethoxyphenyl ketone oxime.--The oxime of 3-thienyl 3, 4-dimethoxyphenyl ketone was prepared according to the procedure of McElvain[21]. The white crystals obtained after recrystallization from ethanol melted at 151-157°.

Anal. Calcd. for $C_{13}H_{14}O_3NS$: S, 12.11. Found: S, 12.46.

Attempted preparation of 3, 4-dimethoxybenzophenone oxime. --The attempted preparation of the oxime of 3, 4-dimethoxybenzophene was done according to McElvain's procedure. All attempts were unsuccessful and the ketone was recovered unchanged.

Attempted preparation of 3-thienyl-3, 4-dimethoxyphenyl methylamine.--3-Thienyl-3, 4-dimethoxyphenyl ketone 2g. (0.0086 mole) was refluxed with 5g. Leuckart reagent for five hours at 165-185°. The reaction mixture was cooled and 2 to 3 volumes of water was added. The oil which separated was refluxed for one hour with 15 ml. concentrated hydrochloric acid. After standing overnight the acidic solution was neutralized with sodium hydroxide and extracted with benzene. After drying over sodium sulfate the benzene was removed by distillation at reduced pressure. The small amount of yellow oil which remained was dissolved in ethanol and picric acid was added. No derivative could be obtained.

SUMMARY

Although many intermediate compounds were prepared, the desired final products were not synthesized. The main reason for this failure was that an olefinic double bond in a molecule containing a thiophene ring could not be reduced satisfactorily.

Catalytic hydrogenation was attempted but was unsuccessful. The reason for the inability of this method to reduce the double bond was probably due to sulfur poisoning by the thiophene ring or possibly due to a small amount of sulfur present as a result of desulfurizing action by the platinum catalyst.

Although many chemical means of reduction were attempted, these methods also proved unsatisfactory. Most of the chemical means tried were too weak to be effective. However, more powerful reducing agents e.g. sodium amalgram, were found to be too drastic and desulfurization was observed.

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INTRODUCTION

In recent years a considerable amount of effort has been devoted to the study of cancer chematherapy using compounds related to mustard gas (di-2-chloroethyl sulfide). The compounds most intensively studied have been the nitrogen mustard derivatives e.g. Methyl di-2-chloroethylamine and tri-2-chloroethylamine. These compounds and others have generally been used intravenously as the water soluble hydrochloride salts.

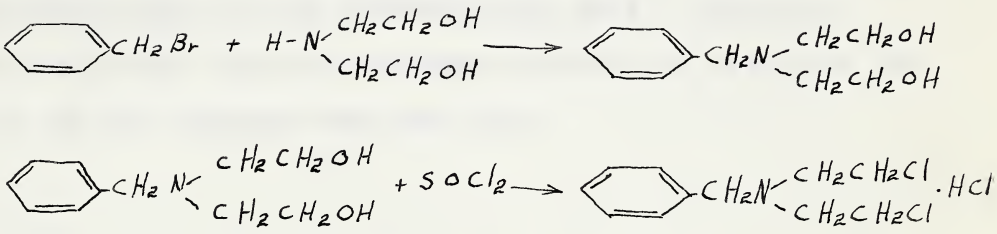
Unfortunately the use of these compounds has been very limited. Results have shown that if too small a dosage was administered very little benefit could be expected. If, however, a larger dosage was given, extensive damage to the bone marrow would result. Although no great success in the field of cancer has come as a result of their use, these compounds have been beneficial to a certain degree.

The main difficulty with the use of nitrogen mustard compounds is in their inherent toxic effects. Recent studies by Campaigne[1] have shown that the replacement of a phenyl group in pharmaceutical drugs e.g.phenolbarbital by a 3-thienyl group resulted in a lowered toxcity. On this basis the preparation of N, N-dichloroethyl-3-thenylamine was attempted. It was hoped that by the introduction of the 3-thienyl group the toxicity of the compound might be lowered sufficiently to render it useful in cancer chematherapy.

For comparison, the corresponding phenyl compound was also prepared. By testing these two compounds it was hoped that the relative toxicity of the 3-thienyl derivative would be obtained.

DISCUSSION

The following sequence of reactions indicates a method of preparing aromatic nitrogen mustard compounds.



This method was followed in the preparation of N, N-dichloroethyl benzylamine and N, N-dichloroethyl-3-thenylamine.

The N, N-diethanolamine compounds were obtained as viscous oils in both cases. Purification of these compounds was done by vacuum distillation and in the case of N, N-diethanol benzylamine, there was no difficulty by this means. However, with N, N-diethanol-3-thenylamine, purification by vacuum distillation (2 mm.) proved unsatisfactory. A disagreeable odor was noticed which seemed to indicate some decomposition. An analysis on the oil obtained gave a sulfur content one percent too high. Hydrochloride and picrate derivatives were made but both were obtained as oils and no analysis was performed on them. This compound was finally characterized by conversion to the dichloro-hydrochloride salt which gave satisfactory analytical data.

The dichloroethyl-hydrochloride salts were obtained in both cases as solid derivatives and were easily purified by recrystallization.

The test animals were injected with one ml. of a solution containing 0.5mg. of the hydrochloride salt. Leucocyte counts were then taken at certain intervals to ascertain the effect of the compound upon the rats.

EXPERIMENTAL

N, N-Diethanol benzylamine.[2]- To a solution of 10.5g. (0.1 mole) diethanolamine in 100 ml. dry benzene was added 12.6g. (0.074 mole) benzylbromide. After refluxing four hours, 16.8g. (0.12 mole) anhydrous potassium carbonate was added. Refluxing was then continued for an additional four hours. After standing overnight the reaction mixture was treated with water to dissolve the excess potassium carbonate. The benzene layer was then separated, washed with water and dried over sodium sulfate. After the benzene layer was evaporated off at atmospheric pressure the residue was vacuum distilled; b.p. 201-202° (20 mm.), yield 11g. (81%),.

N, N-Dichloroethyl benzylamine.--N, N-Diethanol benzylamine 11g. (0.06 mole) was dissolved in 50 ml. dry benzene and to this mixture was carefully added 25g. (0.21 mole) thionyl chloride. The reaction mixture was then carefully refluxed for thirty minutes. After cooling, the benzene and excess thionyl chloride was removed by evaporation under vacuum. The residue was recrystallized from ethanol; m. p. 149-150°, literature m. p. 148-150°[2].

N, N-Diethanol-3-thenylamine.--To a solution of 10.5g. (0.1 mole) diethanolamine was added 17.7g. (0.1 mole) 3-thenylbromide[3] dissolved in 100 ml. dry benzene. After

four hours refluxing, 16.8g. (0.12 mole) potassium carbonate was added then refluxing was continued for an additional four hours. After the reaction mixture had cooled water was added to dissolve the excess potassium carbonate. The benzene layer was then separated, washed with water and dried over sodium sulfate. After the benzene was evaporated off the residue was vacuum distilled; b.p. 140-160° (3 mm.). A benzene insoluble layer from the reaction mixture was also found to contain the desired product and was also purified by a vacuum distillation. Sulfur analyses on the compound, however, were unsatisfactory.

Anal. Calcd. for $C_9H_{15}O_2NS$: S, 15.78. Found: S, 16.92, 17.02, 16.78.

N, N-Dichloroethyl-3-thenylamine hydrochloride.--To 11g. (0.058 mole) N, N-diethanol-3-thenylamine was added carefully 13.04g. (0.11 mole) thionyl chloride. After gentle heating for thirty minutes the excess thionyl chloride was removed at reduced pressure. After the resulting liquid had crystallized, this material was recrystallized from benzene; m. p. 110-111°.

Anal. Calcd. for $C_9H_{14}CL_3NS$: Cl, 38.7 Found: Cl, 38.4.

SUMMARY

N, N-dichloroethyl-3-thenylamine was prepared along with the previously prepared N, N-dichloroethyl benzylamine. Comparative testing of these two compounds is being undertaken at the present time. Preliminary results indicate that both compounds will lower the white blood cell count. No results are available on the relative toxicity of the two compounds.

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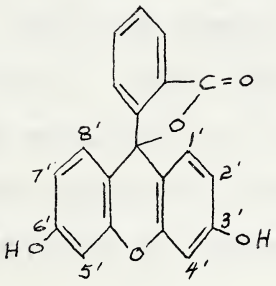
INTRODUCTION

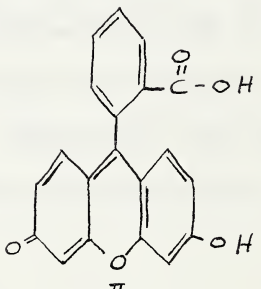
Disubstitution of fluorescein and sulfonefluorescein occurs in the hindered 4', 5'-positions. Sulfonefluorescein does not undergo tetrabromination. Fluorescein in ammonium hydroxide is resistant to tetraiodination. It is suggested that these facts may be explained on the basis of an energetically favorable transition state. It is also suggested that the difference in properties between fluorescein and sulfonefluorescein may be due to the greater nucleophilic character of the carboxylate ion, compared with that of the sulfonate ion.

Orndorff and Hemmer[1] have given the structure of dibromofluorescein as a 4'-5' dibromo derivative. More recently Harris, Marriot and Smith[2] have examined the dibromination of fluorescein and have found that disubstitution occurs in the hindered 4'-5'-positions. Work by Sandin, Gillies and Lynn[3] has substantiated these findings. Moreover the dinitration of fluorescein has also been shown to occur in the 4'-5'-positions.[4]. A very interesting fact is that sulfonefluorescein, unlike fluorescein, does not undergo tetrabromination,[5] and the dibromo compound which it does afford has been shown to be the 4',5'-derivative.[3] It is also of interest that when tetrabromofluorescein dissolved in dioxane and acetic acid is refluxed with excess stannous

chloride-hydrochloric acid reagent, the 4', 5'-dibromo atoms are removed. This is of preparative value in the synthesis of 2', 7'-dibromofluorescein.[3] In order to account for the preference of 4', 5'-over 2', 7'-disubstitution, Sandin, Gillies and Lynn[3] have suggested that the xanthene dyes have a lactoid (I) or quinoid (II) structure in which there is fixation of double and single bonds.



I



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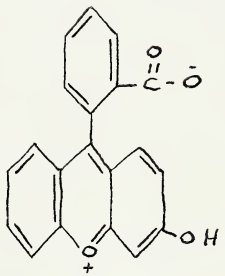


III

The assumption was made that the xanthene dyes resemble xanthone. LeFèvre and LeFèvre[6] had previously determined the dipole moments of xanthone and some xanthone derivatives and had concluded that a nearly complete fixation of double and single bonds occurs in the xanthone skeleton. For that reason it was considered that a fixed structure for fluorescein and sulfonefluorescein was an extension of and in accord with the theoretical prediction of Mills and Nixon[7]:

"that fusion of a benzene ring with a 6-membered ring tends towards the stabilization in the former of the Kekule individual which has a double bond between the points of attachment of the side nucleus." However, there has been much speculation regarding the reality of the Mills and Nixon effect. The structure of fluorescein nevertheless takes on an added interest in view of the recent work of Davies and Jones[8] on the infra-red absorptions of fluorescein and some alkali derivatives. Their results indicate that the classical lactoid structure is to be preferred. They also conclude that the dipolar ion structure (lll) is not the correct representation of fluorescein.

DISCUSSION

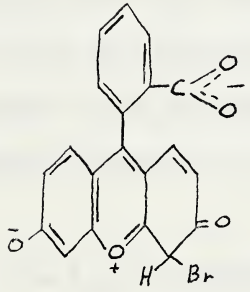
In the present work the iodination of fluorescein with excess iodine in ammonium hydroxide has been examined. In this medium, fluorescein has shown a marked resistance to tetra substitution. The uptake of iodine at the end of 24 hours was 95% of the amount calculated for diiodofluorescein, although the amount of iodine used was sufficient to form the tetraiodo compound.

In the present work there is no rigid proof for the structure of diiodofluorescein. However, on the basis of a similarity between iodination and other electrophilic substitution reactions such as bromination and nitration, the structure is assumed to be 4',5'-diiodofluorescein. Also the fact that substituents in the 2',7'-positions do not hinder diiodination, is evidence in favor of a 4',5'-diodo structure.

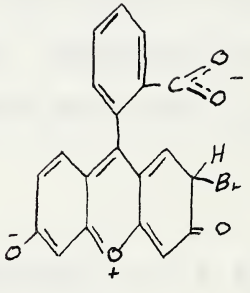
At the end of 7 days the uptake of iodine was 103% of the amount calculated for diiodofluorescein. In a similar manner the compounds 4',5'-dibromo (lV), 2',7'-dibromo- (V), and 2',7'-dichlorofluorescein (Vl) have been treated with iodine. At the end of 7 days, lV showed an uptake of iodine which was approximately 4%. The calculated amount for 4',5'-dibromo- 2',7'-diiodofluorescein is 34.2%. On the other hand, V and Vl showed an iodine uptake which was almost the required amount for diiodination. From these results it is clear that there is a definite resistance to

2',7'-disubstitution.

A plausible explanation for the preferred 4',5'-substitution is the formation of a transition state (Vll) which involves an intact benzene and benzenoid ring and is therefore energetically favorable. The transition state (Vlll) for 2',7'-substitution would be less favorable. It is also suggested that the difference in properties between fluorescein and sulfonefluorescein may be due to the difference in the nucleophilicity of the carboxylate and sulfonate ion.



VII



VIII

EXPERIMENTAL

Fluorescein.--This material was prepared and purified according to the procedure of Orndorff and Hemmer[1].

4',5'-Dibromofluorescein.[9]--To a suspension of 66g. fluorescein in 250 ml. 80% acetic acid was added 64g. bromine at 80°. After two hours of stirring at 80° the product was collected and washed with alcohol and ether. The product was dried at a temperature of 90°. Ten grams of the crude dibromofluorescein was mixed with 40 ml. acetic anhydride and one drop concentrated sulfuric acid. After refluxing for one hour the reaction mixture was poured into water and the diacetate was isolated. Hydrolysis of the diacetate with alcoholic sulfuric acid gave pure 4',5'-dibromofluorescein m. p. 285°.

2', 4', 5', 7'-Tetrabromofluorescein.[1]--To a suspension of 10g. fluorescein in 40g. glacial acetic acid was rapidly added a solution of 28g. bromine in 110g. of glacial acetic acid. After all the fluorescein had dissolved crystals began to separate from the solution. After heating for one hour on a boiling water bath, the mixture was poured into a large volume of water. The crude material was filtered off, dissolved in dilute sodium hydroxide and then precipitated with an excess of acetic acid. The tetrabromo compound was then converted to the diacetate by treatment with acetic

anhydride. The diacetate was saponified by treatment with alcoholic sodium hydroxide. The alcoholic sodium hydroxide solution was distilled to remove a portion of the alcohol. The solution was then acidified with acetic acid, boiled, and a large volume of hot water was added. Upon the addition of concentrated hydrochloric acid, flesh colored crystalls were obtained. Recrystallization from glacial acetic acid yielded the pure colorless tetrabromofluorescein.

2', 7'--Dichlorofluorescein.[10]--Ten grams of phthalic anhydride mixed intimately with 19.7g. monochlororesorcinol was heated to 160° in an oil bath. Five grams of anyhdrous zinc chloride was then added during a period of fifteen minutes. The temperature was then raised to 175-180° and maintained for two and one half hours. After cooling, the reaction product was ground and then boiled for fifteen to twenty minutes in dilute hydrochloric acid and filtered.

The precipitate was dissolved at room temperature in a saturated sodium bicarbonate solution, filtered, heated to boiling and then acidified with dilute hydrochloric acid. After cooling and filtering, the precipitate was refluxed for several hours with acetic anhydride. The diacetate crystallized out upon cooling. The above process was then repeated four times. The diacetate was then hydrolyzed by heating on a water bath for several hours with 85% sulfuric acid. The dichlorofluorescein was then filtered off and dried.

2',7'-Dibromofluorescein.[3]--To a solution of 50g. tetrabromofluorescein in 500 ml. dioxane, 100 ml. concentrated hydrochloric acid and 50 ml. glacial acetic acid was added. 150g. stannous chloride. The solution was refluxed for fifteen to twenty-five hours. At the end of six hours refluxing, crystals of the 2',7'-dibromofluorescein began to settle out and the amount increased as the refluxing continued. After the reaction mixture had cooled, the crystals were filtered off and dissolved in dilute sodium hydroxide. After the solution was filtered the dibromo compound was precipitated by addition of hydrochloric acid. After heating on a water bath the dark red material changed to a bright yellow and became crystalline. This reaction is typical electrophilic aromatic substitution. Bromine is expelled without its bonding electron pair and replaced by hydrogen by the action of the electrophilic reagent hydrogen chloride. Stannous chloride prevents a reversal of the reaction and also bromine transfer. The 4',5'-substitution of bromine can be explained on the basis of a transition state which has a relatively low energy of activation.

Iodination of Fluorescein.--To a solution of 4.0g. (.012 mole) fluorescein in concentrated ammonium hydroxide (250 ml.) was added 12.2g. (0.048 mole) of iodine dissolved in a solution of potassium iodide (25g.) in water (50 ml.).

The reaction mixture was allowed to stand at room temperature for 7 days. During this period the mixture was stirred occasionally and care was taken to prevent the accumulation of explosive compounds on the inside walls of the container. At the end of 24 hours, any solid material had usually dissolved. After 7 days the clear solution was poured with stirring into a mixture of concentrated hydrochloric acid (400 ml.) and ice (1 kg.). After warming gently, the precipitate was filtered off and washed thoroughly with hot water. The solid was dissolved in dilute sodium hydroxide and the precipitation, filtration, and washing procedure repeated. The material was dried and no attempt was made to purify it.

Anal. Calcd. for $C_{20}H_{10}O_5I_2$: I, 43.5. Found: I, 44.8, 44.9.

In similar manner, after 24 hours, the reaction mixture afforded a crude diiodofluorescein which was analyzed.

Anal. Calcd. for $C_{20}H_{10}O_5I_2$: I, 43.5. Found: I, 41.4, 41.3.

Iodination of IV, V and VI.--Compounds IV, V and VI were allowed to react for 7 days with excess iodine in ammonium hydroxide, in the manner described for fluorescein, and the crude products were analyzed. In the case of IV, some iodination took place.

Anal. Found for 0.2086g. $C_{20}H_8Br_2I_2$: Ag halide, 0.1688. The combined AgI and AgBr was converted into AgCl[14].

Found: AgCl, 0.1264g. On this basis the extent of iodination of 4.1%. Calcd. for $C_{20}H_8O_5Br_2I_2$: I, 34.2.

Compounds V and Vl showed an uptake of iodine which was close to the required amount.

Anal. Found for 0.3548g. of $C_{20}H_8O_5Cl_2I_2$: Ag halide, 0.3914g. Found: AgCl, 0.3014g. The extent of iodination was 35.2%. Calcd. for $C_{20}H_8Cl_2I_2$: I, 38.8.

Anal. Found for 0.4034g. of $C_{20}H_8O_5Br_2I_2$: Ag halide, 0.4570g. Found: AgCl, 0.3126g. The extent of iodination was 32.3%. Calcd. for $C_{20}H_8O_5Br_2I_2$: I, 34.2.

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